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BIOCHEMICAL FUEL CELLS

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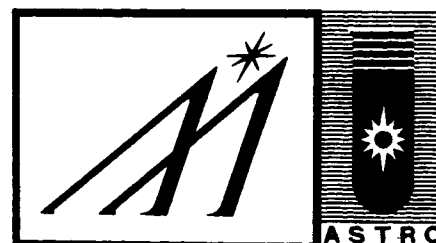
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STUDY OF BIOCHEMICAL FUEL CELLS

(NASA Contract NASw-654) ~~(NASA CR-52813)~~

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ABSTRACT

The purpose of the investigations described in this report was to continue empirical studies on biochemical fuel cells for degrading human wastes and producing electrical energy therefrom. Specifically, the study includes the attachment of organisms to electrode materials, electrode pretreatment and configuration, the selection of suitable organisms and separator materials, selection of electrolytes and additives, structural materials and control devices, and storage and performance characteristics.

During the Second Quarter, investigations have been made of the electrolytic resistivities of all of the electrolytes and separators used in the biofuel cells in this laboratory. It was found that care must be exercised in the use of urine in these tests, because the urine is usually stored for convenience before use, but salts are precipitated and agglomerates form during freezing. If this material is not redissolved and redispersed in the urine by heating gently (120°F) after thawing, the electrolytic resistivity of the previously frozen urine is appreciably higher than that of fresh urine.

Cell constants have also been obtained, to be used in determining the specific resistivities of the electrolytes and separators.

Measurements of the specific resistivities of a limited number of separators and of ion exchange membranes (a total of four) were made. There was only a small difference in the specific resistivities of the materials selected, and so a particular brand of cellulose acetate has been chosen for further studies, for consistency in reporting data. It was chosen for its acceptably low electrolytic resistivity and successful retardation of contamination of anode and cathode media in the cell.

Throughout these studies, consistency in data are being sought wherever possible. For that reason, many variables of the biofuel cell systems are not being studied at the present time. The systems (flow and non-flow) have been standardized with regard to materials and construction of electrodes, separators, cathode media, structural materials, and cleaning and sterilization techniques.

ABSTRACT (Continued)

Reproducibility studies are periodically made of these systems at a set of standardized conditions, and the data obtained are subjected to statistical analysis to prove the invariance of the systems and techniques with time. Reproducibility of the data obtained are generally satisfactory.

Polarization and power curves have been obtained from the flowing system using feces-urine mixtures and the supernatant liquid of a feces-urine mixture, obtained by centrifugation. The maximum peak anodic power density attained with the flowing system has been 1.3 milliwatts per square foot.

Similar polarization and power curves have been obtained from the non-flow system, using feces-urine mixtures in various ratios, either fresh or frozen, with indigenous or added microorganisms, and from vegetarian or omnivorous diets. The maximum peak anodic power density attained from the non-flow system was 4.0 milliwatts per square foot, and a total power density of 9.2 milliwatts per square foot. The anodic open-circuit potentials have ranged from 0.462 volt to 0.719 volt for the various fuel-anolyte mixtures.

Limited literature surveys are continuing on sewage treatment, chemical analysis of urine and feces, and effects of materials on microorganisms.

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STUDY OF BIOCHEMICAL FUEL CELLS

I. Introduction

This report covers the progress attained during the Second Quarter, 1 August through 31 October 1963, in the Study of Biochemical Fuel Cells, Contract No. NASw-654. The purpose of this program is to conduct empirical studies on biochemical fuel cells for producing electrical energy through degradation of human waste.

II. Electrolytic Conductivity and Cell Constants

The impedance bridge and conductivity cell shown in Figure 1 and described in Section VI has been used to determine the cell constants of the various cells, and to measure the electrolytic conductivities of the solutions and separators in use in this laboratory. Some typical resistances of solutions obtained at a frequency of 400 cycles per second are tabulated below. Detailed data are presented in Table I.

<u>Substance</u>	<u>Electrolytic Resistance (ohms)</u>
Deionized water	exceeds 2×10^6
Saturated KCl	3.1
0.1 N KCl	148.8
5% by wt. NaCl + 5% KCl	13.24
Urine, fresh	59.81
Urine, frozen and thawed*	92.2

The above electrolytes represent those currently in use in this laboratory, with the exception of the urine that was frozen and thawed at room temperature. The purpose of that measurement was to determine the effect on the electrolytic conductivity of the urine if this material was not redissolved. Although fresh urine has been used in some of the biofuel cell experiments, the urine is usually frozen and stored for convenience. The frozen urine is not only thawed before use, but is heated gently (120°F) to put the solid particles back into solution; an entire container of urine is thawed and mixed thoroughly before a portion is withdrawn. The removal of the solid particles from urine reduces the electrolytic conductivity of the solution. The temperature to which the urine is reheated is not high enough to be detrimental or to decompose the constituents of urine.

*The urine was thawed at room temperature, but was not heated to redissolve precipitated salts or redisperse agglomerates.

Measurements were also made of the electrolytic resistances of various types of separator materials. The investigation was not intended to be extensive at this time, and was limited to one anion exchange membrane, one cation exchange membrane, one dialysis membrane, and one form of cellulose acetate. The specific resistivities of the separators are summarized below, and detailed data are presented in Table II.

<u>Separator</u>	<u>Specific Resistivity (ohm-cm.)</u>
Anion exchange membrane	33.6
Cation exchange membrane	47.2
Dialysis membrane	79.6

Only erratic results have been obtained when the resistivity of the cellulose acetate was measured.

Measurements of the resistivities of the separators were made in the conductivity cell shown in Figure 1; the cell has an internal diameter of 40 millimeters and the distance between electrodes is approximately 10 inches.

Another property of separators that is of primary importance in the biochemical fuel cell, and which is also being evaluated in this laboratory, is the ability of the separator to retard the contamination of the electrolytes caused by mass diffusion through the membrane, both chemically and bacteriologically. On this basis, the dialysis membrane above and listed in Table II is not used in the present studies; although it presents a relatively low electrolytic resistance, it was found to allow gross contamination by diffusion in a relatively short period of time.

Cell constants were obtained, using an oscilloscope (Tektronix Type 502 Dual Beam) with the impedance bridge shown in Figure 1. The electrolyte used was saturated potassium chloride in deionized water. Detailed data are given in Table III, and sample calculations are presented in Appendix A.

III. Polarization and Power Curves

Polarization and power curves were obtained for the pumping and non-pumping systems. In general, the details of these systems are as follows:

A. Physical Description

1. Flowing System

Cell: Plastic (Lucite) (see Figures 2 and 3)

Electrodes: Platinized screen (90% Pt - 10% Rh), 80 mesh, 0.003 in. diameter wire, 2-1/8 in. clear diameter, 3.54 sq. in. geometric area).

Separator: Cellulose acetate (Sargent S-14825, 0.001 in. thick).

O-Rings: Silicone (Dow Corning S-7180).

Catholyte: 5 wt. % NaCl - 5% KCl in sterile, deionized water; non-biological; bubbled with purified, gaseous oxygen.

Water-Proof and Chemically Resistant Paint: Temprotec TP220 Red (Ryan Herco Products Corp., Burbank, California).

Fuel-Anolyte: Composition varies; see individual experiments for details.* Mixture was homogenized (Coronet Blender, manufactured by Iona Mfg. Co., Manchester, Conn.) and bubbled with gaseous helium. When frozen urine was used, it was thawed and gently heated (120°F) to redissolve the precipitated salts. Feces was obtained in most cases from a collection obtained from volunteers on a low-cellulose diet; the sample was frozen for storage immediately after collection.

2. Non-Flow System

Cell: Glass, H-shape, O-ring type (see Figure 4).

Electrodes: Platinized platinum foil, 1 sq.in. area (non-opposing faces coated with water-proof and chemically resistant paint).

Separator, O-Rings, Catholyte, Fuel-Anolyte, and Water-Proof and Chemically Resistant Paint: Same as for flowing system.

Detailed data regarding these experiments may be found in Table IV.

*For consistency in comparing data, the concentration of the fuel-anolyte has usually been 30 grams feces in 100 milliliters urine.

B. Data

1. Flowing System

a. Urine-Feces: Polarization and power curves were obtained with the flowing system, as shown in Figures 5 and 6. The fuel-anolyte was 30 grams human feces in 100 milliliters fresh, human urine. A peak anodic power density of approximately 1.3 milliwatts per square foot was obtained. (Run 1)

b. Urine-Feces Diluted: A mixture was made of 30 grams human feces in 100 milliliters fresh, human urine. The mixture was homogenized and bubbled with helium gas. The peak anodic power density was 0.15 milliwatts per square foot and that of the total cell was 0.22 milliwatts per square foot. The mixture was diluted (10%) with sterile, deionized water to retard foaming. (Run 2)

c. Supernatant Liquid: A mixture was made of 30 grams human feces in 100 milliliters fresh, human urine. After mixing and homogenization, the material was centrifuged at 5000 rpm. for 20 minutes. The supernatant liquid was diluted 1:1 with sterile, deionized water to determine the effectiveness of this concentration as a biofuel. A peak anodic power density of 0.33 and a total cell power density of 0.59 milliwatts per square foot were obtained. (Run 3)

2. Non-Flow System

a. Urine-Feces with Escherichia Coli: Polarization and power curves were obtained from the system employing E. coli in a feces-urine mixture prepared as described in Section IV, Phase V, of the Reproducibility Study. The peak total power density was 9.0 milliwatts per square foot and the anodic power density 1.2 milliwatts per square foot; the short-circuit current density was 25 milliamperes per square foot. (Run 6)

b. Urine-Feces Supernatant with Escherichia Coli: A fuel-anolyte mixture was prepared as described in Phase III of the Reproducibility Study, Section IV. The peak anodic power density was 4.0 milliwatts per square foot and the peak total power density 9.2 milliwatts per square foot. (Run 4)

c. Lyophilized Frozen and Fresh Frozen Feces: For storage purposes, the lyophilization of feces may be desirable if the biochemical and electrochemical activity of the feces are not affected detrimentally. A test was conducted to compare the biofuel characteristics of lyophilized feces with that of fresh frozen feces. Two H-cells were assembled as described in Section III.A.2; two more were assembled in which lyophilized feces was used. The concentration of lyophilized feces was 8 grams in 100 milliliters urine, which

corresponds to the concentration of 30 grams of whole feces in 100 milliliters urine used in the two cells described above (whole feces is approximately 75% water by weight). The feces was lyophilized after homogenizing in distilled water; lyophilization from a feces-urine mixture was not successful.

The open-circuit potential of the lyophilized material varied from +0.100 volt (saturated calomel electrode reference) to -0.625 volt over a period of 70 hours, and approached that of the fresh frozen feces; however, this value was attained by the fresh frozen feces after only about 5 hours. (Run 5)

d. Fresh and Lyophilized Fresh Feces: An experiment was conducted to compare the biofuel value of fresh feces with that of lyophilized fresh feces. The fresh feces fuel-anolyte was prepared as described in Section III.A.2. The lyophilized fresh feces was initially prepared in a similar manner, then lyophilized as described in Section III.B.2.c; the weight of lyophilized feces used in the experiment was 8 grams, corresponding to 30 grams of whole feces. The fresh feces was obtained from a volunteer on a simulated astronaut's diet, low in cellulose. (Run 7)

The peak anodic power density of the lyophilized fresh feces-urine was 0.21 milliwatts per square foot and the peak total power density 0.42 milliwatts per square foot; the peak anodic power density of the fresh feces-urine was 1.5 milliwatts per square foot.

Because of the reduction in power of the lyophilized feces as compared to the fresh feces, an experiment was run to determine whether any of the material removed during lyophilization is electrochemically active. The material removed while lyophilizing feces in a concentration of 30 grams fresh feces in 100 milliliters distilled water was thawed and used as a fuel-anolyte. This system displayed an anodic open-circuit potential of -0.146 volt (versus saturated calomel) as its best value, but insufficient current was obtained to make a polarization plot. Therefore, the reduction in power was not due to electrochemically active substances in the frozen lyophilized material.

Lyophilized feces was obtained from another company conducting similar types of research. The feces was lyophilized in a commercial freeze drier. The lyophilized feces was then mixed with urine, as described for the study of lyophilized material in Section III.B.2.c, to provide comparable data. Duplicate tests were run. The initial pH of this mixture was 6.7; the best anodic open-circuit potential was -0.641 volt; the peak anodic power density was 4.1 milliwatts per square foot, at an anodic open-circuit potential of -0.600 volt; and a short-circuit current density of 72 milliamperes per square foot was obtained. The reproducibility of this system is shown in Figure 7.

e. Feces-Urine Ratio: Earlier in this program, studies were made of the effects of feces-urine ratios. However, these results were based only on open-circuit potentials and instantaneous values of potentials and currents obtained at various values of the external resistance. The results of these tests are summarized below:

Run No. 8	I	II	III	IV
Gms. feces/100 ml. urine	0	10	15	25
Anodic open-circuit potential at time of polarization study (volt)	-0.220	-0.585	-0.385	-0.630
Peak anodic power density (mw./sq.ft.)	0.022	0.95	0.26	1.4
Short-circuit current density at time of polarization study (ma./sq.ft.)	2	13	6	36

This general study was repeated to verify the previous results, but at slightly different concentrations than those reported above. Results of this experiment are tabulated below:

Run No. 9	I	II	III	IV
Gms. feces/100 ml. urine	10	20	30	40
Anodic open-circuit potential at time of polarization study (volt)	-0.638	-0.564	-0.488	-0.469
Peak anodic power density (mw./sq.ft.)	3.4	1.85	0.7	0.5
Short-circuit current density at time of polarization study (ma./sq.ft.)	30	26	24	22

These data indicate that the optimum feces-urine ratio seems to be between 10 and 20 grams feces per 100 milliliters urine, disregarding the probably erroneous data obtained at a concentration of 15 grams feces per 100 milliliters of urine.

An interesting aspect of this study is the reversal of anodic open-circuit potential with time. The order of potentials early in the experiment is reversed later in the experiment; e.g., after 20 hours, the anodic potentials increase with increasing feces concentration; whereas after 62 hours, the anodic potentials decrease with increasing feces concentration.

f. Comparison of Urine with the Supernatant Liquid from a Feces-Urine Mixture: A test was conducted to compare the electrochemical power output of urine with the supernatant liquid obtained by centrifuging a feces-urine mixture, prepared as described in Section III.A., at 5000 revolutions per minute for 20 minutes. The data are compared below:

<u>Description</u>	<u>Urine</u>	<u>Supernatant Liquid of a Urine-Feces Mixture</u>
pH, Initial	5.7	6.6
pH, Final	8.9	8.8
Best anodic open-circuit potential (volt)	-0.545	-0.687
Anodic open-circuit potential at time of polarization study (volt)	--	-0.603
Peak anodic power density (mw./sq.ft.)	--	0.06
Short-circuit current density at time of polarization study (ma./sq.ft.)	--	19

Insufficient current was obtained from the urine biofuel alone to permit a polarization study. The initial concentration of the urine-feces mixture was 30 grams feces in 100 milliliters urine.

g. Comparison of Vegetarian and Omnivorous Feces: An experiment was conducted with the fuel-anolyte mixture prepared as described in Section III.A., but using feces from a vegetarian in substitution for feces from an omnivorous diet. The data are tabulated in Table IV, where the peak anodic power density is reported as 1.6 milliwatts per square foot. (Run 10)

IV. Reproducibility

The reproducibility of data obtained from the biochemical fuel cells has been a matter of primary interest throughout this program. For that reason, a set of experimental conditions has been established for both the flow and non-flow systems, as described in Section III.A.

The reproducibility of data is established by making reproducibility testsevery three or four runs. These tests are not time-consuming and last approximately 24 hours. They provide valuable information in verifying that the system is the same and invariant with time, and in eliminating questions regarding the effects of possible contamination and of changes in sources of materials or in techniques that vary over an extended period of time.

The reproducibility tests were made in several phases, to determine whether chemical and bacteriological complexities affected the reproducibility. The phases are described below:

- Phase I Escherichia coli in brain heart infusion
- Phase II Feces-urine supernatant, E. coli, and brain heart infusion
- Phase III Feces-urine supernatant, with less E. coli and brain heart infusion than in Phase II
- Phase IV Feces-urine mixture with only indigenous microorganisms
- Phase V Feces-urine mixture with centrifuged E. coli

In Phase I, the fuel-anolyte was as simple, and hopefully as invariant, as possible. This system employed a single microorganism (fresh Escherichia coli) in a pure nutrient (brain heart infusion). The E. coli culture was added to sterile brain heart infusion media to provide a mixture containing 10% by volume of E. coli culture and 90% media. The E. coli was grown in brain heart media. Four cells were assembled, the test covered a period of approximately 65 hours, and the reproducibility was acceptable, as shown by the data in Table V. The data in Table V reflect the worst conditions; i.e., the maximum differences in open circuit potentials of the four cells at any given time.

Phase II demonstrated the reproducibility of a fuel-anolyte similar to that of Phase I but containing the supernatant liquid of a feces-urine mixture. The original feces-urine mixture was prepared as described in Section III.A. This mixture was centrifuged at 5000 rpm for 20 minutes at 15°C. A portion of the supernatant liquid (50 milliliters) was mixed with 40 milliliters sterile brain heart infusion and 10 milliliters fresh E. coli culture (also in brain heart infusion). Again, four cells were prepared, the test lasted approximately 16 hours, and the reproducibility was satisfactory, as shown by the data in Table V.

Phase III was next, and the fuel-anolyte was essentially the same as in Phase II but with a lower concentration of E. coli and brain heart infusion media. In this phase, $\frac{1}{2}$ milliliter of E. coli culture was grown in 500 milliliters brain heart infusion, and 10 milliliters of this inoculum was added to 90 milliliters of the human waste supernatant. Again, four essentially identical cells were prepared, the test lasted approximately 80 hours, and the reproducibility was satisfactory.

In Phase IV, the feces-urine mixture was employed as the fuel-anolyte, with no additions of microorganisms or nutrient medium. The mixture was the same as that described on p. 3. This test lasted approximately 18 hours, and the reproducibility of the four cells assembled was satisfactory. The reproducibility of this system is shown in Figure 9 and in Table V.

In Phase V, the reproducibility was determined of a fuel-anolyte to which an external microorganism was added, but essentially without accompanying nutrient. An E. coli suspension in brain-heart infusion was centrifuged at 5000 rpm for 20 minutes; the supernatant liquid was removed, and the E. coli was suspended in 1 milliliter sterile, deionized water. The resuspended E. coli (1 milliliter) was added to the feces-urine mixture, which was prepared as described in Section III.A. Four similar cells were prepared, the test lasted approximately 50 hours, and the results were satisfactory. The reproducibility became poorer with time, and one of the cells appeared to introduce broader variations than the other three cells. These variations are shown on the next page.

	<u>Four cells, 50 hours</u>	<u>Four cells, 24 hours</u>	<u>Three cells, 24 hours</u>
Variance	423	89	12.6
Standard deviation	11.1	9.4	3.55
Mean deviation	± 16	± 8	± 3
% Error	43.2	30.7	25.0
Mean	37	26	12

The pH of all cells was 8.7 initially, and 8.4-8.6 at the completion of the test.

V. Microbiological Aspects

The developmental aspects of biochemical fuel cell work require that only a limited microbiological research program be conducted. Indigenous organisms must be accepted, since there is no practical means at present of sterilizing human waste in space travel.

The indigenous organisms will grow according to their biochemical capabilities and under the given nutrient and physical conditions of the fuel cell. The complex nature of the metabolic reactions can be recognized in view of the number of different organisms which might grow, the variation in numbers of any type of organisms which may be eliminated in human waste, the changes associated with different diets, human physiological variance in intestinal digestion of food, and microbiological variations such as mutation and synergistic and antagonistic reactions between microbes.

The supply of human feces used in experiments described in this report was obtained by combining the fecal output of several apparently healthy individuals on a special low-cellulose diet. The feces was then frozen and stored for convenience. The electrochemical activity and power output of these mixtures has been measured, primarily at room temperature and without additives. Thus, the electrochemical activity is related to indigenous microbial metabolism.

Theoretically, the most desirable system would be one stabilized by the presence of one or a few types of microorganisms in large numbers and yielding satisfactory electrochemical activity. Since many of the microbes

present in feces at the time of elimination by the human will not grow at room temperature or thrive without nutrients or changes in conditions, the microbial flora will change significantly as the feces decompose under indigenous attack. This microbial change may consume considerable time without significant electrochemical advantage. Thus, the desirable system should have large numbers of electroactive microbes to enhance power output. The described situation may be accomplished by (1) inoculating useful microbes into feces at the start of decomposition, or (2) inoculating relatively small quantities of fresh feces into relatively large quantities of actively decomposing feces, as in typical sewage treatment. At this time it cannot be stated that either system will be preferred for the biochemical fuel cell, but a limited study will be conducted to evaluate the differences in the two techniques.

The earlier efforts will be based upon the addition of single organisms in large quantities to urine-feces mixtures. There are several sources of these organisms: (1) Contractors in an essentially parallel type of biochemical fuel cell research, (2) related research in this laboratory, and (3) outside sources. One of the contractors engaged in similar research has recently provided a sample of Bacillus pasteurii for evaluation in this laboratory.

Organisms from the indigenous mixture in feces and urine are being isolated by adding 3% agar (in a 1.5% salt solution) to an equal volume of urine-feces and incubating plates anaerobically and aerobically. A number of organisms have grown moderately well on the surface of agar as isolated colonies. Colonies of the microbes were transferred to nutrient broths; growths in broths were plated on trypticase soy agar. Isolated colonies, examined microscopically for purity, were transferred to sterilized urine agar and urine-feces agar plates to affirm growth on these nutrients. Thus, the purity of the cultures initially isolated was established.

Several Gram positive rods (Bacillus species by morphology and culture), several Gram negative rods, and one Gram positive coccus have been isolated thus far. Precise microbiological identification will not be performed unless an organism shows significant electrochemical activity in the fuel cell. The vigorous growth of one of the above Gram positive rods, which does not obligatively require urea, makes it a likely candidate for further work.

Inocula of Bacillus pasteurii and of the microbial candidates considered in this laboratory to be the most promising for fuel cell activity will be tested.

VI. Apparatus and Equipment

A continuous flow system is illustrated in Figure 2. Two plastic cells are illustrated, connected so that the anolyte and catholyte flasks are common to both. The 5-neck flasks provide for gas bubblers, gas release tube, pH adjustment, nutrient addition, withdrawal of media to be pumped through the cells and returned to the 5-neck reservoirs, and stirring by means of magnetic stirrers. The cells are constructed of acrylic plastic (Lucite), and the tubing of Tygon. The pumps are located in the center of the photograph. The cells may be clamped-off from each other when taking electrical measurements. In the foreground is a voltmeter, an ammeter, and a decade resistance box.

A dismantled plastic cell is illustrated in Figure 3. From the left in the photograph, are shown a solid end plate, a screen electrode with water repellent and chemically resistant paint to prevent leakage, two electrode reservoirs (one for anolyte and one for catholyte), a second screen electrode, and a second end plate, respectively. A separator, such as a cellulose acetate or ion exchange membrane (not shown) would be placed between the electrolyte reservoirs. The tubes on opposite sides of the reservoirs provide flow into and out of the cell, while threaded nylon rods are passed through the holes at each corner to hold the cells in place. The plastic cell is 3 inches square and has electrodes 1-5/16 inches apart, with an exposed diameter of 2-1/8 inches. It is constructed of 80-mesh platinized platinum screen.

The non-flow system is illustrated in Figure 4. The figure illustrates a glass H-cell, consisting of tubing about 7-3/4 inches long by 1-1/8 inch I.D. and has a horizontal distance of 2-1/2 inches between vertical tubes. The two halves of the cell are joined with O-ring joints, which are held together with a clamp. The electrodes are platinized platinum foil, with 1 square inch area on each side, approximately 3-1/2 inches apart. Gas bubblers are provided for bubbling gaseous helium and oxygen (or air) over the anode and cathode, respectively. A Luggin capillary (salt bridge) provides measurements of potentials of one electrode (the anode) versus a reference electrode (saturated calomel).

Many static cells may be used in screening experiments, so that comparisons of several separator and electrode materials may be conducted simultaneously. A digital printout box will accommodate up to 50 cells, reading voltage or current at prearranged time intervals.

VII. Future Work

During the next quarter, investigations will be conducted in the following areas:

- A. Limited studies regarding the addition of enzymes to feces-urine mixtures to form specific products.
- B. Comparison of a direct biofuel cell with an indirect one; i.e., the comparison of the biofuel cell activities of systems wherein the biological and electrochemical sites are common as contrasted to those in which they are separated.
- C. Galvanometric and coulometric studies of feces-urine fuel cells to determine the total amount of energy available.
- D. Bomb calorimetric studies to determine heats of combustion of dried feces.
- E. Potentiostatic tests of biofuel cells containing feces-urine mixtures.
- F. Continued reproducibility and polarization studies.
- G. Pretreatment of feces-urine mixtures chemically and bacteriologically.
- H. Limited chemical analyses of feces-urine mixtures after various time intervals in biofuel cells.
- I. Continued studies of effects of materials and of effectiveness of sterilization techniques in the biofuel cell investigations.

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APPENDICES

APPENDIX A

CELL CONSTANTS

CALCULATIONS OF CELL CONSTANTS

The cell constants were calculated from the following equations:

$$L = \frac{Kd}{A}$$

$$L = \frac{\Lambda c}{1000}$$

$$L = JK = J/R = \frac{d}{AR}$$

$$L = 1/k$$

L = specific conductance of the solution (mho/cm.)

k = specific resistance of the solution (ohm-cm.)

K = conductivity of the solution (mho)

d = distance between electrodes (cm.)

A = cross section of electrolytic path (sq.cm.)

Λ = equivalent conductance of solution (mho/equivalent/sq.cm.)

c = concentration of solution (equivalents/liter)

J = cell constant (cm.⁻¹)

R = measured resistance (ohms)

Equivalent conductivities (Λ) and specific conductivities (L) of various electrolytes may be obtained from several sources (1-4), and the specific resistance (k) can be calculated from them. For example, the specific resistance of saturated potassium chloride, used in obtaining the cell constants tabulated in Table III, is 2.45 ohm-cm. Using this value and measuring the resistances of the various cells, the data of Table III were obtained.

The technique used in making these determinations was that of four-electrode conductometry. Briefly, in this procedure four electrodes were used; two platinized platinum foil electrodes were inserted in the conductivity cell shown in Figure 1 to carry the current, and two saturated calomel reference electrodes were placed between the two platinum electrodes. The conductivity cell is relatively long and narrow to provide essentially parallel electrolytic paths. The reference electrodes were placed at the upper edge of the platinum electrodes to avoid shielding.

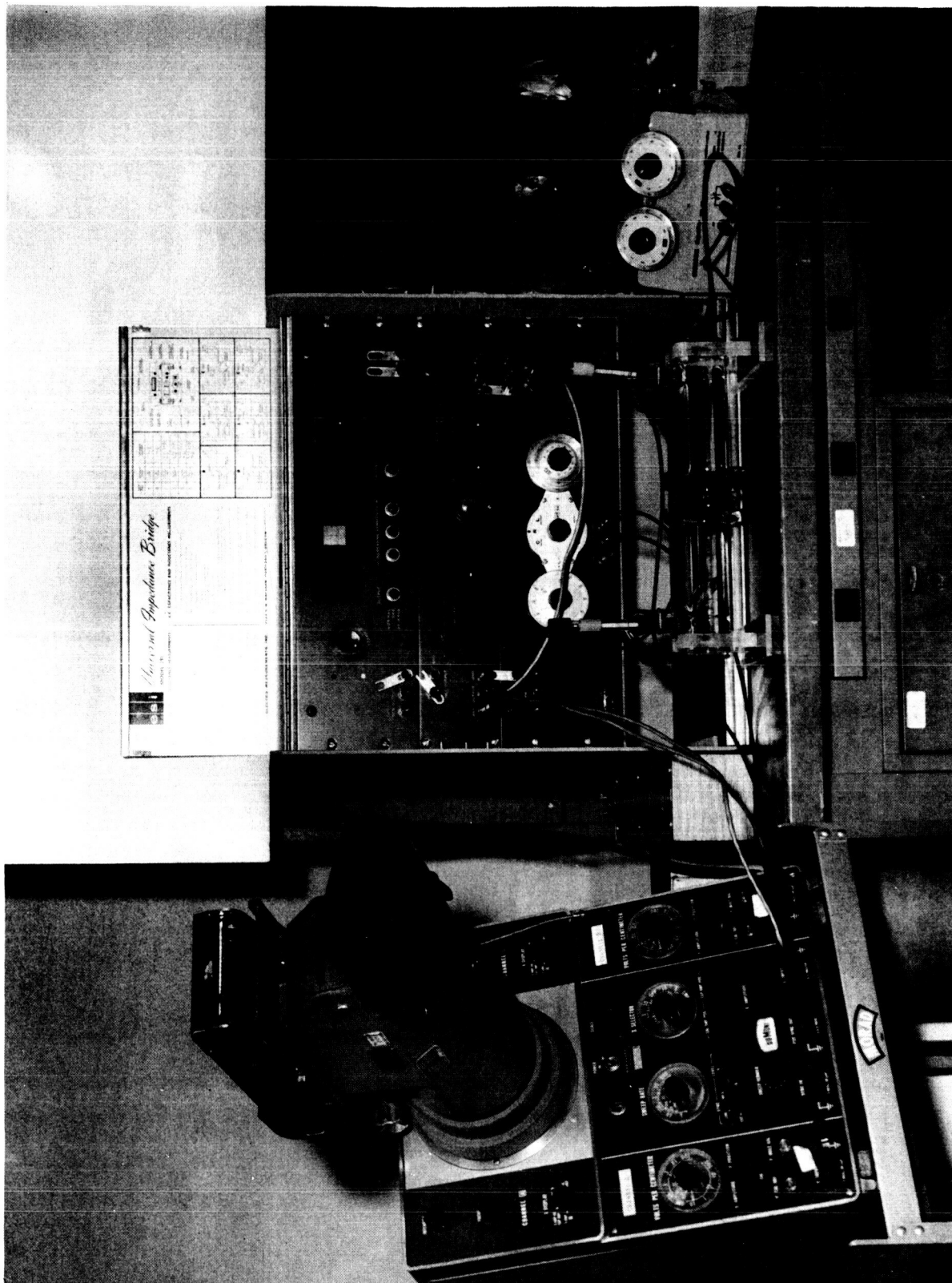
APPENDIX B

DATA AND APPARATUS

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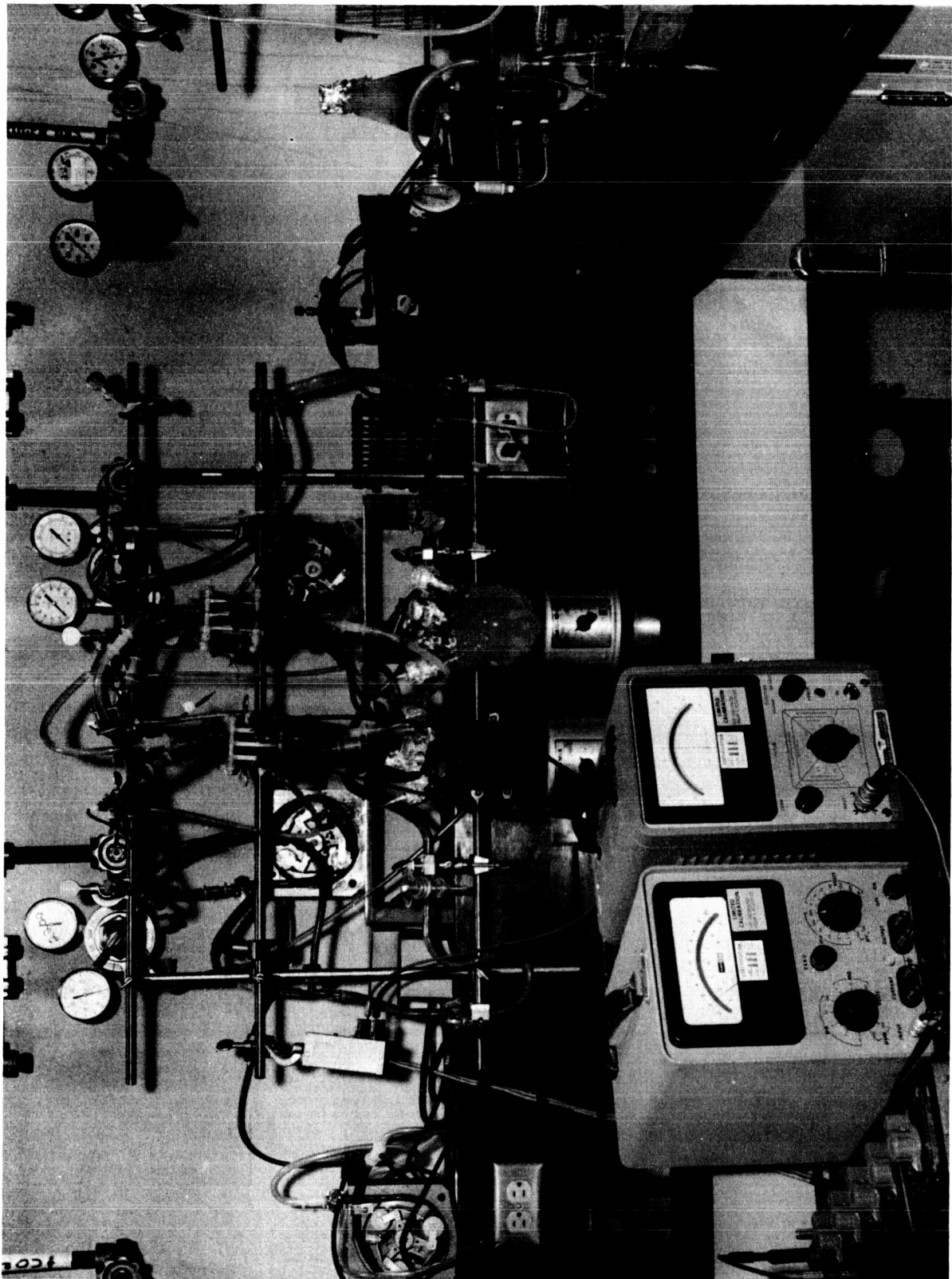


A-C CONDUCTIVITY MEASUREMENTS EQUIPMENT

UNCLASSIFIED

UNCLASSIFIED

BIOFUEL CELL. CONTINUOUS FLOW SYSTEM



R-16,215
Neg. 4805-1

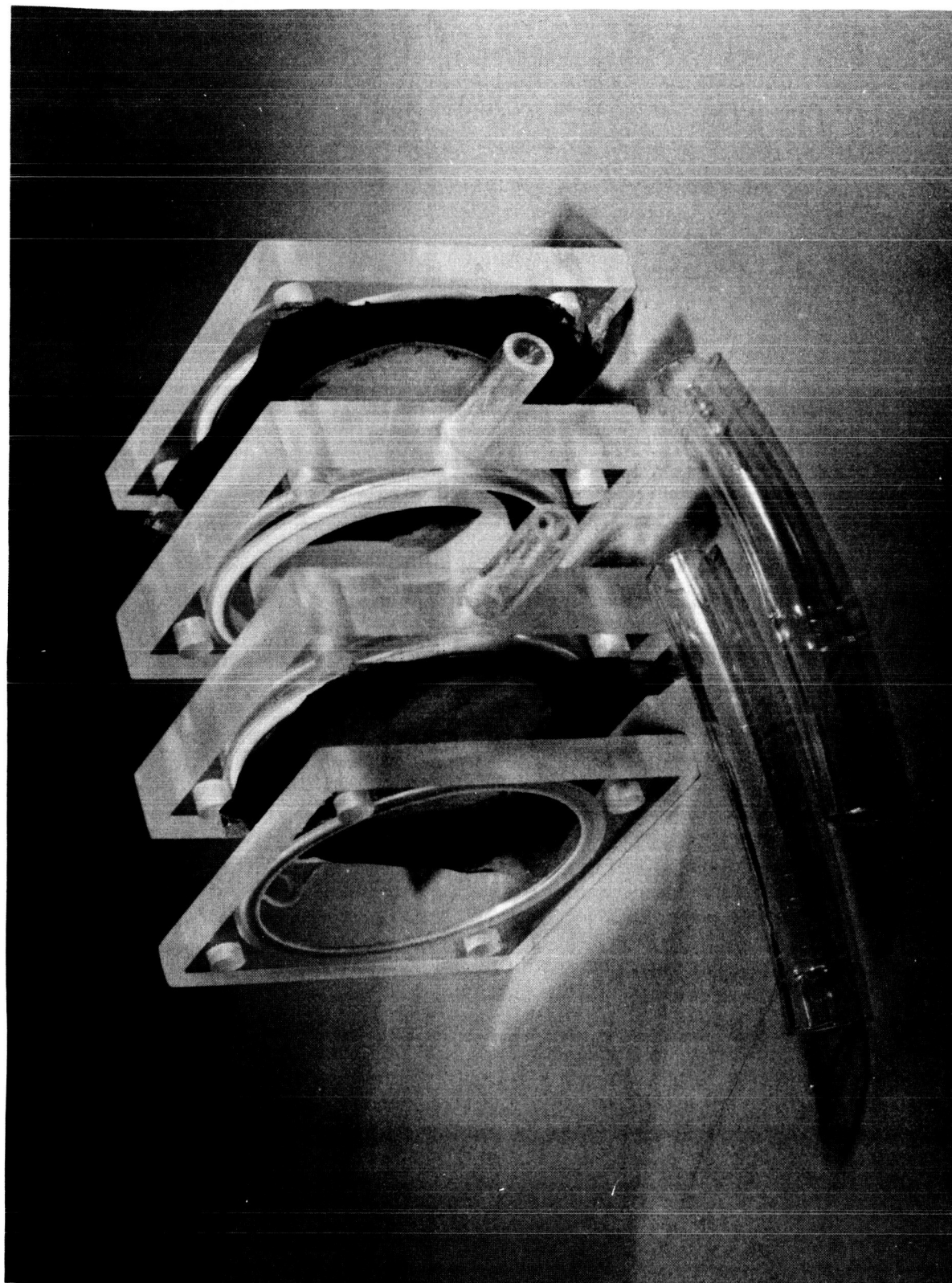
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Figure 2

UNCLASSIFIED

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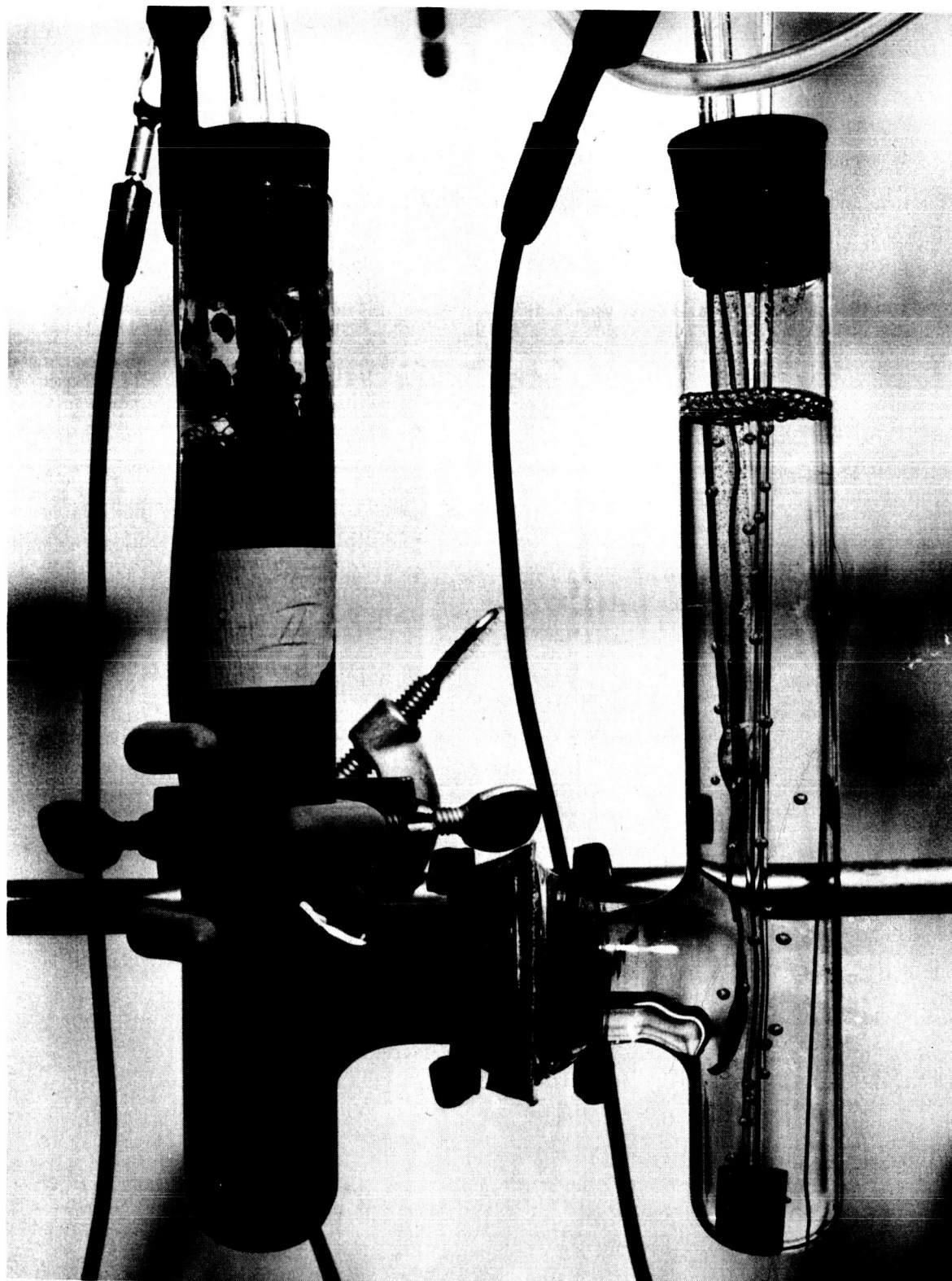
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PLASTIC CELL, DISMANTLED

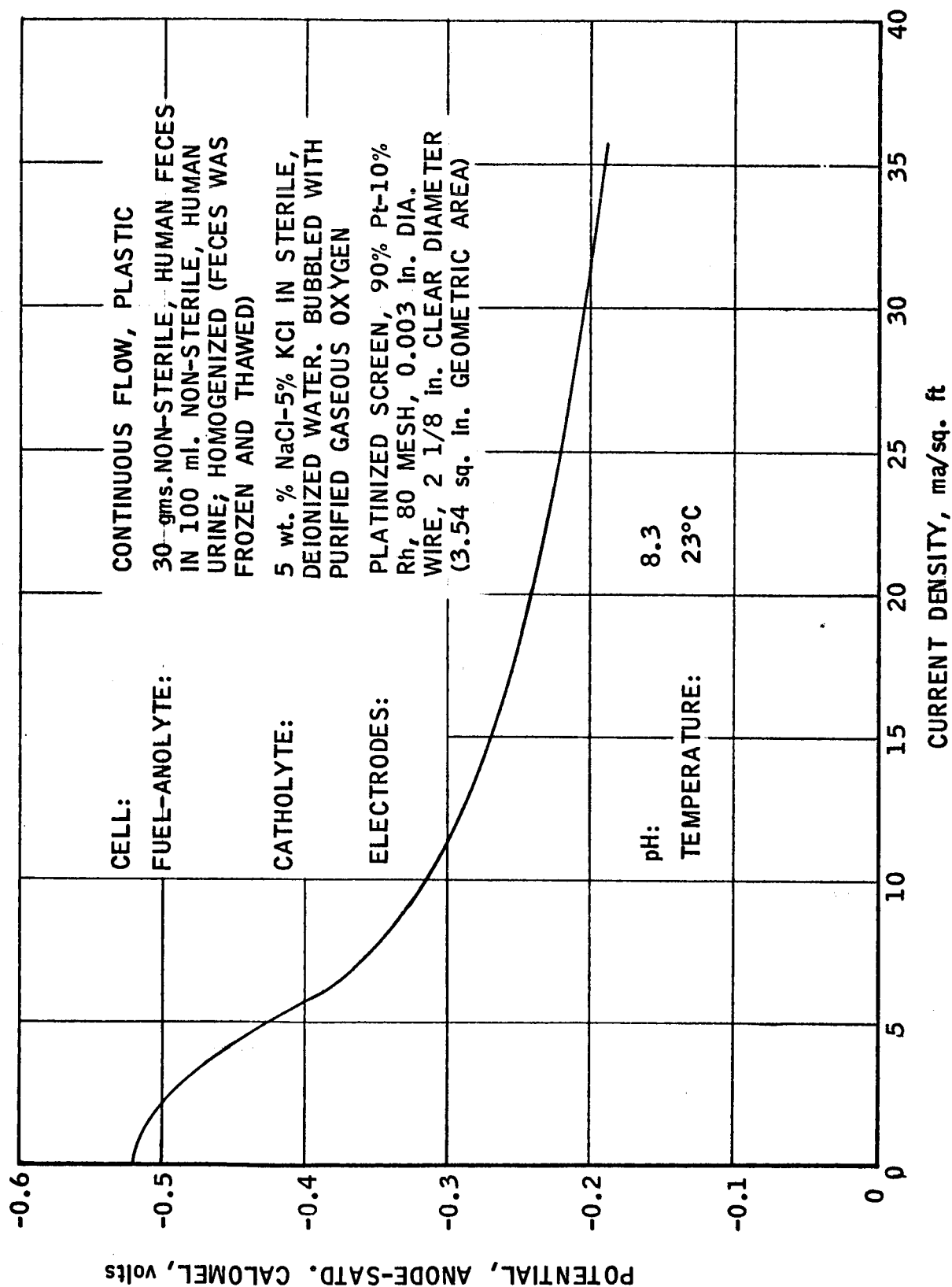
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H-CELL; O-RING JOINT TYPE (CLOSEUP)



R-16,217
Neg. 4831-3

ANODIC POLARIZATION



ANODIC POWER DENSITY

CELL: CONTINUOUS FLOW, PLASTIC
ELECTRODES: PLATINIZED SCREEN, 90% Pt - 10% Rh, 80 MESH, 0.003 in. DIA. WIRE, 2 1/8 in. CLEAR DIAMETER (3.54 sq. in. GEOMETRIC AREA)
O - RINGS: SILICONE
SEPARATOR: CELLULOSE ACETATE
CATHOLYTE: 5 wt. % NaCl - 5% KCl IN STERILE, DEIONIZED WATER; BUBBLED WITH PURIFIED GASEOUS OXYGEN
FUEL-ANOLYTE: 30 gms. NON-STERILE, HUMAN FECES, FROZEN AND THAWED, IN 100 ml. FRESH, NON-STERILE, HUMAN URINE. HOMOGENIZED. BUBBLED WITH GASEOUS HELIUM.
pH: 8.3
TEMPERATURE: 23°C

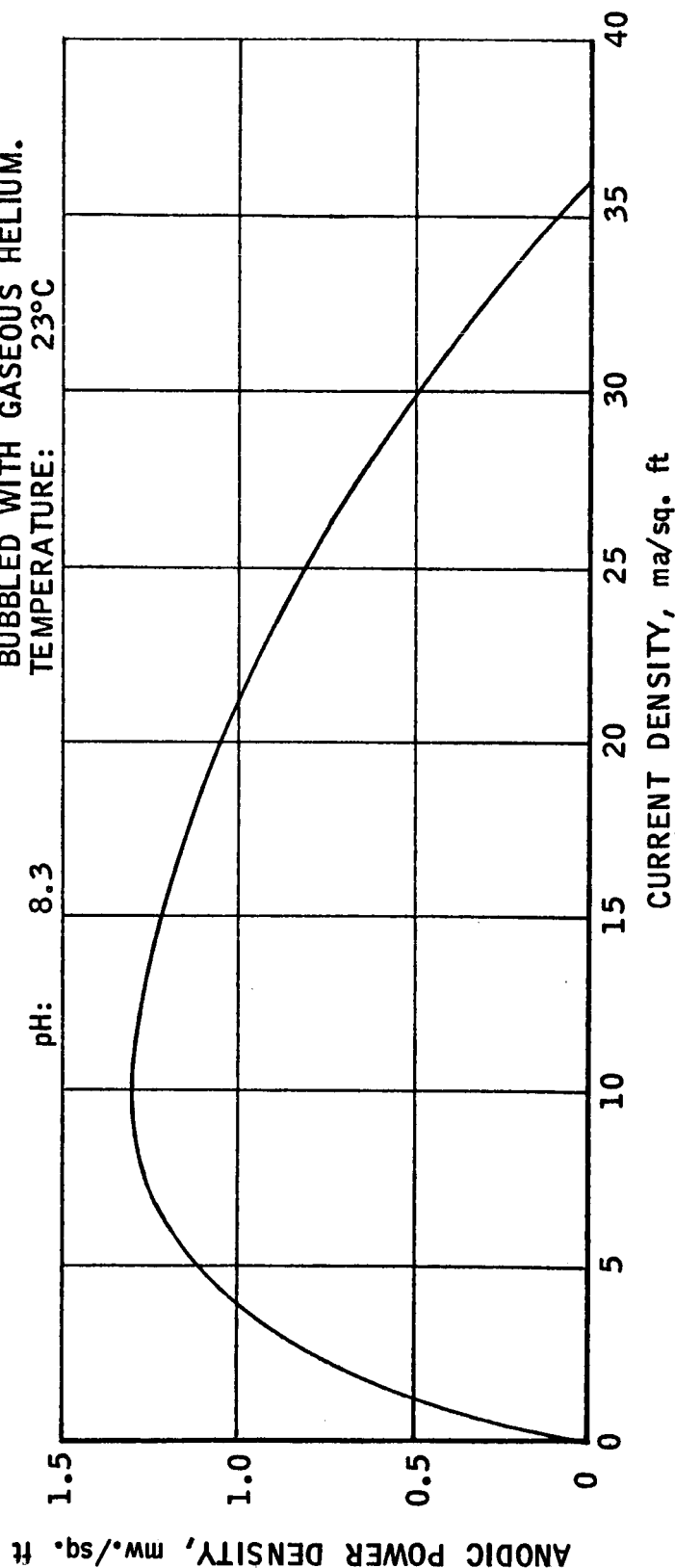


Figure 6

REPRODUCIBILITY STUDY USING LYOPHILIZED FECES

CELL: H-CELL, GLASS, O-RING TYPE,
NON-PUMPING

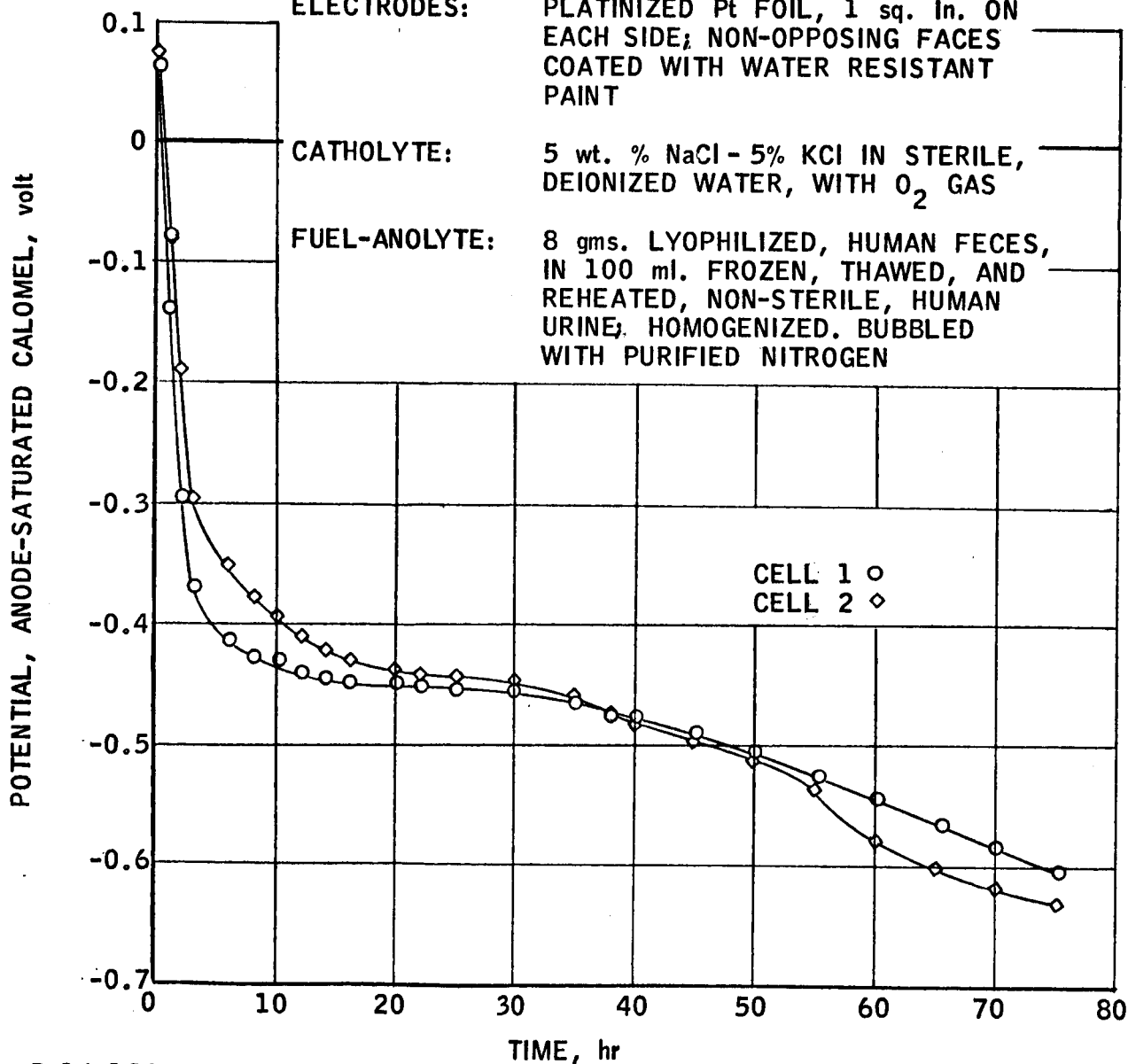
SEPARATOR: CELLULOSE ACETATE

O-RINGS: SILICONE

ELECTRODES: PLATINIZED Pt FOIL, 1 sq. in. ON
EACH SIDE; NON-OPPOSING FACES
COATED WITH WATER RESISTANT
PAINT

CATHOLYTE: 5 wt. % NaCl - 5% KCl IN STERILE,
DEIONIZED WATER, WITH O₂ GAS

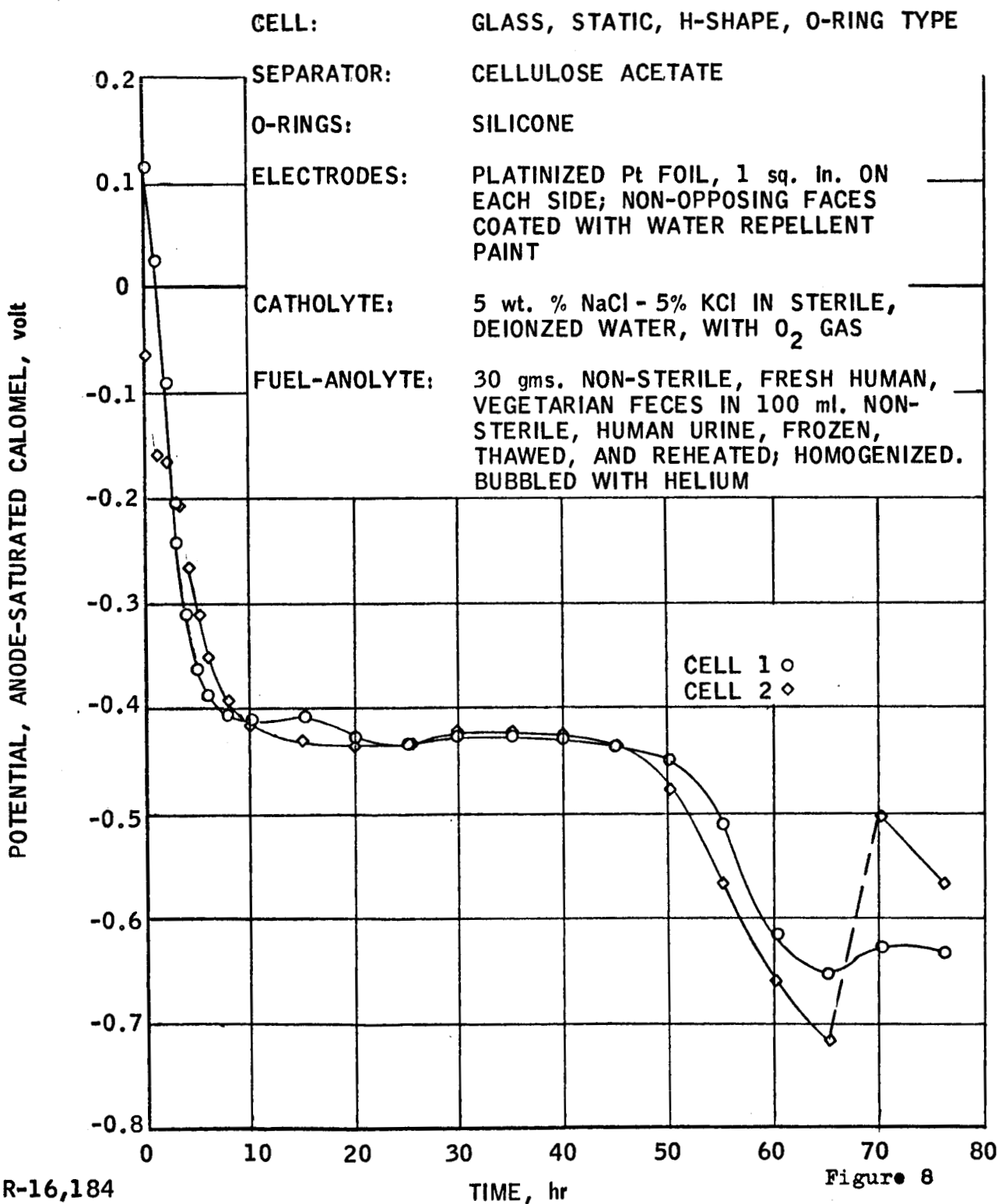
FUEL-ANOLYTE: 8 gms. LYOPHILIZED, HUMAN FECES,
IN 100 ml. FROZEN, THAWED, AND
REHEATED, NON-STERILE, HUMAN
URINE; HOMOGENIZED. BUBBLED
WITH PURIFIED NITROGEN



R-16,186

Figure 7

ANODIC POLARIZATION VEGETARIAN FECES



REPRODUCIBILITY STUDY URINE-FECES WITH INDIGENOUS MICRO-ORGANISMS

CELL: H-CELL, GLASS, O-RING TYPE,
NON-PUMPING

SEPARATOR: CELLULOSE ACETATE

O-RINGS: SILICONE

ELECTRODES: PLATINIZED Pt FOIL, 1 sq. in. ON
EACH SIDE; NON-OPPOSING FACES
COATED WITH WATER RESISTANT
PAINT

CATHOLYTE: 5 wt. % NaCl - 5% KCl IN STERILE,
DEIONIZED WATER, WITH O₂ GAS

FUEL-ANOLYTE: 30 gms. NON-STERILE, HUMAN FECES
IN 100 ml. NON-STERILE, HUMAN
URINE; HOMOGENIZED. BOTH FECES
AND URINE WERE PREVIOUSLY FROZEN
AND THAWED

CELL 1 ○
CELL 2 ◇
CELL 3 □
CELL 4 △

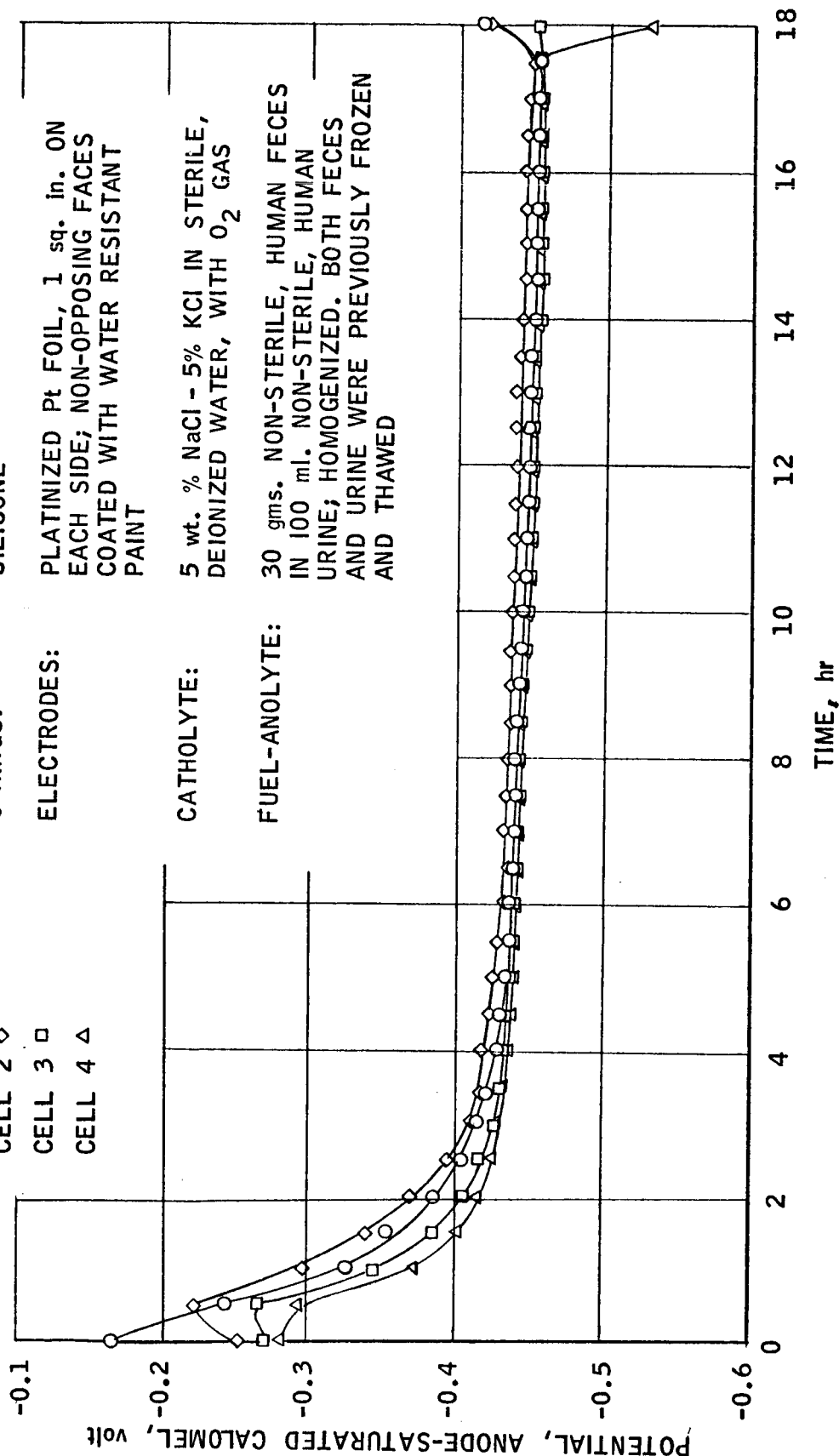


Figure 9

TABLE I

ELECTROLYTIC RESISTIVITIES OF SELECTED ELECTROLYTES (ohms)
(Each value is an average of five readings)

	<u>First Test</u>		<u>Second Test</u>		
	<u>Frequency (cps)</u>		<u>Frequency (cps)</u>		
	<u>400</u>	<u>1000</u>	<u>400</u>	<u>1000</u>	<u>10,000</u>
Saturated KCl	--	--	3.1	3.4	3.4
0.1 N KCl	143.7	145.2	148.8	148.4	145.3
2.5% NaCl by weight + 2.5% KCl	23.59	23.56	23.34	23.06	--
5% NaCl by weight + 5% KCl	13.21	13.21	13.24	13.56	13.48
Frozen urine, filtered	94.05	93.53	--	--	--
Fresh urine	59.81	59.10	--	--	--
Urine, frozen then thawed at room temperature	92.2	95.3	92.2	95.3	96.0
Deionized water	Exceeds 2×10^6				

TABLE II

ELECTROLYTIC RESISTIVITY OF SEPARATORS (ohms)
(Each value is an average of five readings)

	First Test		Second Test		Avg. Resistance of Separator at 400 cps (ohms)	Specific Resistivity (ohm-cm.)
	Frequency (cps)	10,000	Frequency (cps)	400		
Satd. KCl, after soaking separators	400	1000	3.316	5.409	5.199	--
Cellulose acetate in the above satd. KCl	2976*	2435*	497*	1913*	1730*	--
Anion exchange membrane in the above satd. KCl	3.360	3.575	3.814	5.478	5.363	33.6
Cation exchange membrane in the above satd. KCl	3.56	3.66	3.92	5.408	5.296	47.2
Same satd. KCl, after tests	3.31	3.45	3.53	5.416	5.274	--
Satd. KCl, after soaking dialysis membrane	5.240	5.178	--	--	--	--
Dialysis membrane in the above satd. KCl	5.285	5.202	--	--	.045	79.6
Same satd. KCl, after tests	5.251	5.179	--	--	--	--

Cellulose acetate was E.H. Sargent Co. S-14825, 0.001 in. thick (.0025 cm.)
Anion exchange membrane was Ionics, Inc., Nepton AR-111A, 0.024 in. thick (.061 cm.)
Cation exchange membrane was Ionics, Inc., Nepton CR-61, 0.024 in. thick (.061 cm.)
Dialysis membrane was VanWaters and Rogers, Inc., No. 25225, 0.0028 in. thick (.0071 cm.)

*These values are believed to be excessively high, but the reason for them has not been determined.

Cross-sectional area of all membranes = 12.6 sq.cm. (40 mm. I.D.)

Deionized water was used to make the electrolytes.

TABLE III
CELL CONSTANTS

Electrolyte: Saturated KCl solution

Temperature: 25°C

	<u>Frequency (cycles/sec)</u>	<u>Resistance (ohms)</u>	<u>Cell Constant (cm.⁻¹)</u>
Conductivity	400	5.223	2.13
	1000	5.047	2.06
H-Cell, O-Ring Type	400	4.931	2.01
	1000	4.895	1.99
H-Cell, Sintered	400	4.847	1.98
Plug Type	1000	4.734	1.93
H-Cell, Sintered	400	4.912	2.00
Plug with Agar	1000	4.804	1.96

TABLE IV

POLARIZATION AND POWER DATA

A. Flowing System

Run No.	1	2	3
Peak Anodic Power Density (mw./sq.ft.)	1.3	0.33	0.15
Peak Total Power Density (mw./sq.ft.)	-	0.59	0.22
Short Circuit Current Density (ma./sq.ft.)	36	11	4
Open Circuit Anodic Potential at time of polarization study (volt)	0.591	-0.435	-0.460
Best Anodic Open Circuit Potential (volt)	0.591	-0.475	-
pH, Initial	-	-	-
pH, Final	8.3	7.9	-
Duration of Test (hours)	68	144	

TABLE IV

POLARIZATION AND POWER DATA (continued)

B. Non-Flow System

Run No.	4	5-frozen	5-lyophilized	6	7-lyophilized	7-fresh
Peak Anodic Power Density (mw./sq.ft.)	4.0	3.0	2.0	1.2	.21	1.5
Peak Total Power Density (mw./sq.ft.)	9.2	4.8	3.6	9.0	.42	1.5
Short-Circuit Current Density (ma./sq.ft.)	105	73	42	25	5	21
Open-Circuit Anodic Potential at time of polarization study (volt)	-0.660	-0.65	-0.625	(Approx.) -0.626	-0.45	-0.60
Best Anodic Open-Circuit Potential (volt)	-	-0.65	-0.625	-0.626	-0.663	-0.691
pH, Initial				8.7	6.9	6.8
pH, Final		8.5	6.6	8.4-8.6	-	-
Duration of Test (hours)	-	-	-	50	140	140

TABLE IV

POLARIZATION AND POWER DATA (continued)

B. Non-Flow System (cont.)

Run No.	8-I	8-II	8-III	8-IV	9-I	9-II	9-III	9-IV
Peak Anodic Power Density (mw./sq.ft.)	0.22	0.95	0.20	1.4	3.5	1.85	0.7	0.5
Peak Total Power Density (mw./sq.ft.)	.04	1.35	0.26	2.7	3.9	2.0	0.8	0.7
Short-Circuit Current Density (ma./sq.ft.)	2	13	6	36	30	26	24	22
Open-Circuit Anodic Potential at time of polarization study (volt)	-0.220	-0.585	-0.385	-0.630	-0.638	-0.564	-0.488	-0.469
Best Anodic Open-Circuit Potential (volt)	-0.463	-0.635	-0.465	-0.535	-0.642	-0.697	-0.579	-0.608
pH, Initial	5.75	-	8.20	8.20	6.5	6.7	7.0	7.5
Duration of Test (hours)	208	208	208	208	184	184	184	184

TABLE IV

POLARIZATION AND POWER DATA (continued)

B. Non-Flow System (cont.)

Run No.	10	11-I	11-II
Peak Anodic Power Density (mw./sq.ft.)	1.6	-	-
Peak Total Power Density (mw./sq.ft.)	2.3	-	-
Short-Circuit Current Density (ma./sq.ft.)	33	-	-
Open-Circuit Anodic Potential at time of polarization study (volt)	-0.638	-	-
Best Anodic Open-Circuit Potential (volt)	-0.719	-0.652	-0.462
pH, Initial	7.6	7.1*	6.95
Duration of Test (hours)	89	254	254

*pH of sterile portion; non-sterile portion was 6.95

TABLE V

STATISTICAL DATA FOR REPRODUCIBILITY TESTS

Phase	I	II	III	IV	V
Mean maximum difference (millivolts)	16.49	20.36	40.50	9.04	37
Mean deviation of maximum differences	9.58	10.213	31.84	.96	3.0
Standard deviation of maximum differences	11.63	18.09	37.44	1.19	3.55

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